*Carbohydrate Research*, 148 (1986) C1–C4
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands



**Preliminary communication**

# X-Ray fibre diffraction results from *Alcaligenes* (ATCC 31555) microbial polysaccharide S-130 and a comparison with gellan gum

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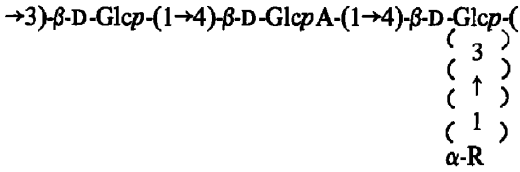
(Received December 20th, 1985; accepted for publication, February 3rd, 1986)

The bacterium *Alcaligenes* (ATCC 31555) secretes an extracellular anionic polysaccharide[1–3] (S-130 or Biozan) which is currently produced commercially using aerobic submerged-fermentation procedures. Aqueous dispersions of S-130 have high viscosities at low-shear rates and exhibit shear-thinning behaviour. The high value of the viscosity is maintained at elevated temperatures and the polysaccharide has potential industrial applications as a thickening agent and for supporting particulates in suspension[1–3].

S-130 is a branched polysaccharide[4–6] with the following structure:




where R is either L-Rha or Man. The Man residue was originally reported[4] to be D, but subsequent results[5,6] supported its being L. To our knowledge, an α-L-Man residue has not been reported previously in a bacterial polysaccharide. The ratio of terminal rhamnose to terminal mannose is ~2:1, and ~50% of the repeat units carry an *O*-acetyl group[5]. It is not known whether the distribution of rhamnose and mannose residues is random or whether they are attached to the backbone in clusters, or whether there is a mixture of two polysaccharides one with Rha side-chains and the other with Man side-chains. The backbone of S-130 may be visualised as the trisaccharide β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc sandwiched between α-L-Rha residues. This would be anticipated to be a relatively stiff trisaccharide segment, by analogy with cellulose, flanked by rather flexible glycosidic linkages.

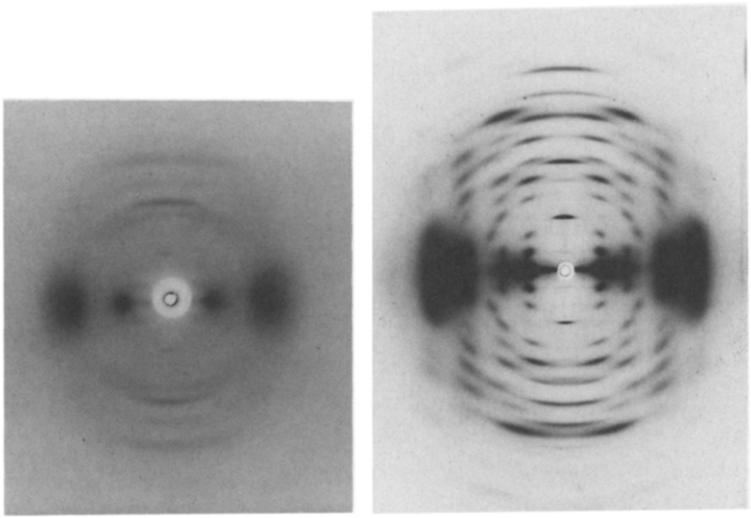
The backbone of S-130 is identical to that of gellan gum[7,8], the anionic linear polytetrasaccharide secreted by *Pseudomonas elodea*. Thus, X-ray fibre diffraction data on the conformation of S-130 are of interest, not only for their own sake, but also for com-

0008-6215/86/$03.50 

parison with the data for gellan gum[9—12] the relevant features of which are summarised below.

Gellan gum is an effective gelling agent in aqueous dispersion. The native form contains, on average, one *O*-acetyl group per repeating unit, which is believed to be located at position 6 of one or both glucose residues[8]. The native form produces weak, elastic gels and the deacetylated gum yields hard, brittle gels[3]. The X-ray fibre diffraction patterns[9—12] obtained from oriented samples of acetylated gellan gum exhibit three-fold helical symmetry with an axial advance per structural repeat-unit of 0.94 nm, a value less than half the 2 nm expected for a linear tetrasaccharide repeating-unit. It has not been established whether gellan gum forms contracted single helices or extended, and intertwined, parallel double helices in the condensed state[10,11]. Deacetylation of gellan gum causes enhanced intermolecular association and a substantial improvement in crystallinity (see Fig. 1) which appears to be correlated with increased brittleness of the gels. The diffraction signals index onto a trigonal unit-cell with parameters $a = b = 1.56$ nm, $c$ (fibre axis) = 2.82 nm.

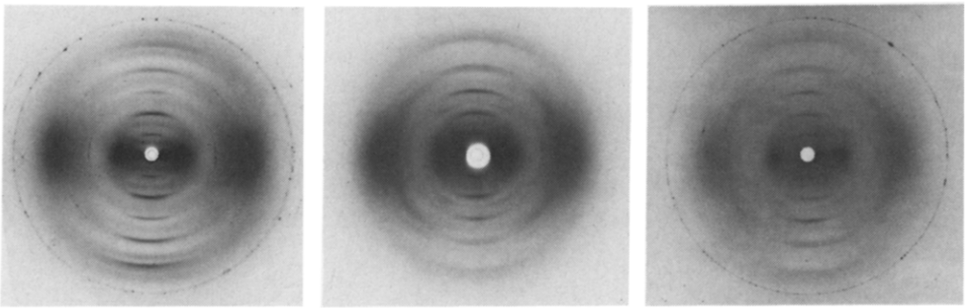
The addition of a single saccharide branch per tetrasaccharide repeating-unit, which is the basic difference between gellan gum and S-130, causes a fundamental change in the rheology of the aqueous dispersions. S-130 exhibits good thermal stability, coupled with high viscosity at low shear rates and shear-thinning behaviour, but does not gel, whereas gellan gum forms thermoreversible gels[3]. In an attempt to account for these differences, S-130 has been studied by X-ray fibre diffraction.



(a) (b)

Fig. 1. X-Ray fibre diffraction patterns from oriented fibres of gellan gum: (*a*) native material, (*b*) deacetylated material. The fibre axis is vertical.

Samples of S-130 were purified[6], and oriented fibres and films suitable for X-ray fibre diffraction were prepared[13]. Cast films of S-130 could be oriented by stretching, with typical extensions of 150%. Figure 2a illustrates the X-ray fibre diffraction pattern obtained for the native acetylated S-130. The meridional diffraction signals occur on even layer lines as orders of a spacing of 1.83 nm. The simplest explanation is a two-fold helical conformation, with a pitch of 3.66 nm and an axial advance per structural repeat-unit of 1.83 nm. The X-ray pattern shows that the molecules are reasonably aligned in the stretch direction, but the broadness of the equatorial signals and the general lack of crystallinity indicate poor lateral packing and interchain register.



(a) (b) (c)

Fig. 2. X-Ray fibre diffraction patterns from oriented fibres of polysaccharide S-130 at 100% relative humidity: (*a*) native material; meridional signals (on vertical bisector) occur on even layer lines at spacings which are orders of 1.83 nm (first meridional) and are interpreted as a two-fold extended ribbon; (*b*) deacetylated material, taken on an Elliott rotating-target X-ray generator; (*c*) deacetylated sample, taken using the S.E.R.C. Daresbury synchrotron source.

The value of 1.83 nm correlates with the expected value of 2 nm for a tetrasaccharide and is suggestive of an extended ribbon-like chain. Computer modelling of the S-130 chain with two-fold symmetry confirms that such a conformation is stereochemically possible[14]. This finding is in contrast to the value of 0.94 nm for gellan gum.

Since deacetylation of gellan gum improved the crystallinity substantially[9,11,12], S-130 was deacetylated by treatment with alkali (0.2M NaOH, 4°) and the reaction was monitored by loss of the i.r. band at 1730 $cm^{-1}$. Oriented fibres were then prepared, and X-ray fibre diffraction patterns were obtained by using a rotating-target X-ray generator (Fig. 2b) and the S.E.R.C. Daresbury synchrotron source (Fig. 2c). The pattern shows no significant change in the crystallinity, chain conformation, or interplanar spacings. Thus, unlike gellan gum, the association and interaction of polysaccharide S-130 chains is not seriously influenced by acetyl groups.

The poor lateral associations and interactions between polysaccharide S-130 chains preclude gel formation. It remains to be established if the ordered form of S-130 is retained in aqueous dispersion and whether the stability of the ordered structure can explain the retention of high viscosity at elevated temperatures.

ACKNOWLEDGMENTS

We thank the A.F.R.C. and the S.E.R.C. for support, and Dr. R. Moorhouse (Kelco division of Merck & Co.) for samples of gellan gum and S-130.

REFERENCES

1 K. S. Kang, G. T. Veeder, and I. W. Cottrell, *Prog. Ind. Microbiol.*, 18 (1983) 231–253.
2 J. K. Baird, P. A. Sandford, and I. W. Cottrell, *Biotechnology*, (1983) 778–783.
3 P. A. Sandford, I. W. Cottrell, and D. J. Pettit, *Pure Appl. Chem.*, 56 (1984) 879–892.
4 M. A. O'Neill, R. R. Selvendran, and V. J. Morris, *Int. Carbohydr. Symp., XIIth, Utrecht*, 1984, Abstr. B2.4.
5 P.-E. Jansson, B. Lindberg, G. Widmalm, and P. Sandford, *Carbohydr. Res.*, 139 (1985) 217–223.
6 M. A. O'Neill, R. R. Selvendran, V. J. Morris, and J. Eagles, *Carbohydr. Res.*, 147 (1986) 295–313.
7 M. A. O'Neill, R. R. Selvendran, and V. J. Morris, *Carbohydr. Res.*, 124 (1983) 123–133.
8 P.-E. Jansson, B. Lindberg, and P. A. Sandford, *Carbohydr. Res.*, 124 (1983) 135–139.
9 V. Carroll, G. R. Chilvers, D. Franklin, M. J. Miles, V. J. Morris, and S. G. Ring, *Carbohydr. Res.*, 114 (1983) 181–191.
10 C. Upstill, E. D. T. Atkins, and P. T. Attwool, *Int. J. Biol. Macromol.*, in press.
11 P. T. Attwool, E. D. T. Atkins, C. Upstill, M. J. Miles, and V. J. Morris, in G. O. Phillips, D. J. Wedlock, and P. A. Williams (Eds.), *Gums and Stabilisers for the Food Industry. 3*, Elsevier Applied Science, London, 1986, pp. 135–145.
12 V. Carroll, M. J. Miles, and V. J. Morris, *Int. J. Biol. Macromol.*, 4 (1982) 432–433.
13 E. D. T. Atkins, C. F. Phelps, and J. K. Sheenan, *Biochem. J.*, 128 (1972) 1255–1263.
14 K. Veluraja, E. D. T. Atkins, and P. T. Attwool, unpublished data.